Remarks

Claims 1-3, 7, and 16-23 were pending prior to this Response. By the present communication, no claims have been added or canceled, and claim 22 has been amended to define Applicants' invention with greater particularity. Support for the amended claims may be found throughout the specification and claims as filed. Accordingly, upon entry of the present amendment, claims 1-3, 7, and 16-23 will be pending in this application.

Objection to the Claims

The Office Action points out that claim 22 contains a typographical error. Without acquiescing to the reasoning offered by the Office, and in order to expedite prosecution of the instant application, Applicants have amended claim 22 as suggested by the Examiner. Withdrawal of the objection is respectfully requested.

Rejection under 35 U.S.C. § 103

Applicants respectfully traverse the rejection of claims 1-3, 7, and 16-23 under 35 U.S.C. §103(a) as allegedly being unpatentable over Giuliani, *et al.* J. Exp. Med. April 1998, Vol. 187, No. 7, 1123-1132 (hereinafter "Giuliani") in view of Esposito, *et al.* Infection and Immunity, July 1970, Vol. 2, No. 1, 120-22 (hereinafter, "Esposito"). The U.S. Supreme Court decision in the *KSR International v. Teleflex Inc.* (82 USPQ2d 1385), modified the standard for establishing a *prima facie* case of obviousness. Under the *KSR* rule, three basic criteria are considered. First, some suggestion or motivation to modify a reference or to combine the teachings of multiple references still has to be shown. Second, the combination has to suggest a reasonable expectation of success. Third, the prior art reference or combination has to teach or suggest all of the recited claim limitations. Factors such as the general state of the art and common sense may be considered when determining the feasibility of modifying and/or combining references.

The Office Action asserts that "Contrary to Applicant's argument, the motivation is clearly provided by Giuliani et al. Giuliani et al. established that LTR72 has residual toxicity. Thus, while Giuliani et al. may note that it is likely that LTR72 has the appropriate safety

window to be safely used in the open population, including adults and children, however, this statement is merely that of the opinion of the author for it is not substantiated by any data." (Office Action, page 3).

Applicants respectfully disagree with the Examiner's position that the safety window of LTR72 described in the reference is subjective and merely the opinion of the author. Giuliani clearly cites data of the residual toxicity of LTR72, stating that LTR72 has 100,000-fold lower toxicity than wild-type LT in Y1 cells *in vitro* (see Abstract of Giuliani). Further studies surrounding the efficacy of LTR72 at baseline, *i.e.* non-attenuated, toxicity of the same have been conducted by various research groups, all of which are strongly indicative that LTR72 is well-tolerated in latent form and does not require attenuation to render it less toxic (PubMed Abstracts of Ward et al., Infect Immun 1999, 67(10): 5124-32 [Exhibit A]; Tierney et al., J Infect Dis. 2003, 188(5): 753-8 [Exhibit B]; and Kende et al., Vaccine 2007, 25(16): 3219-27 [Exhibit C]; and Chen et al., Vaccine 2002, 20(21-22): 2671-9 [Exhibit D]). Moreover, according to a LT bacterial toxin study performed by Tamura et al. (PubMed Abstract of Jpn J Infect Dis 2000, 53(3): 98-106 [Exhibit E]) the safety window for LT derivatives is such that "the toxicity of the derivatives, as determined by the Y-1 adrenal cell assay, should not exceed 1/100 EC(50) of the native [toxin]."

The Office Action further alleges that Giuliani itself questions the safety of LTR72 and cites the following sentence from the reference: "[t]he question is whether LTR72 mutant can be safely used in humans." The Office Action draws a conclusion based on the citation that "it would have been prima facie obvious for one of ordinary skill in the art to further detoxify LTR72, thereby rendering it nontoxic for use in humans."

Applicants respectfully submit that from this cited passage, a skilled artisan would not have been motivated to attenuate or "further detoxify" the LTR72 mutant disclosed by Giuliani upon consideration of the context of the entire passage (cited only in part by the Action), which reads as follows,

> The question is whether LTR72 mutant can be safely used in humans. So far the experience in humans is limited; CTB has been safely used at a 1-mg oral dose and 100 μg intranasal dose. In the case of LT, the only published information refers to the use of LTG192. This was safe orally at 5-, 25-, and 50-μg doses,

> whereas, at a 100-μg dose it induced mild diarrhea in some of the volunteers. Based on the above data, it is likely that LTR72, which in the rabbit ileal loop is at least 20-fold less toxic than LTG192, can be safely used in humans at high doses. Since the immunogenic and adjuvant dose is predicted to be in the range of 25-200 μg, it is likely that LTR72 has the appropriate safety window to be safely used in the open population, including adults and children. (Giuliani, page 1128-1129, right and left columns, respectively in under the heading "Discussion").

The question posed in the reference regarding the safety of LTR72 in humans is merely hypothetical, serving only to segue into a discussion of the safety of other toxins and ultimately leads the author to the conclusion that LTR72 would indeed have the appropriate safety window to be used in the human population. The paragraph as a whole does not disclose or suggest the necessity of further attenuation of LTR72. Thus, the Action's allegation that one of ordinary skill would have been motivated to modify LTR72 is not supported by the disclosure of Giuliani or other research groups, as illustrated above. The observation that LTR72 has residual toxicity alone is not sufficient to motivate one of ordinary skill in the art to further detoxify LTR72. A skilled artisan would consider the disclosure of Giuliani et al. as a whole and/or other art-recognized findings based on the research of LT derivatives as potential adjuvants *e.g.*, LTR72 is an excellent mucosal adjuvant that can be safely used, a high level of detoxification reduces adjuvanticity, and existing LTK63 is nontoxic yet has lower adjuvanticity than wild-type LT and LTR72.

The Office Action further alleges that according to Giuliani, "[LTK63 along with LTR72] are excellent mucosal adjuvants" and further posits that "in the instant case, Giuliani et al. clearly demonstrates that a reasonable expectation of success can readily be ascertained."

Applicants respectfully submit that LTK63 and LTR72 are genetically attenuated toxins that have not been treated with formalin. Since the residual toxicity of LTR72 is sufficiently low, it would not be predictable whether formalin treatment would detoxify the already attenuated LTR72 without affecting its adjuvanticity. Exposure of LTR72, and all other proteins, to formalin results in the reaction of lysine with formaldehyde (formalin is an aqueous solution of formaldehyde). LTR72 is genetically attenuated and the lysine residues in the same are free

and not bound to formaldehyde; binding of the lysine residues in a protein would affect the secondary and tertiary structures and necessarily the characteristics of the protein/toxin, which would have far-reaching consequences not restricted to toxicity alone. Accordingly, one of ordinary skill in the art would not have a reasonable expectation of success at arriving at the claimed invention by modifying LTR72 with the method disclosed by Esposito.

The Office Action alleges that "[a]t the time the invention was made, Esposito et al. teaches the detoxification of toxins using formalin at a temperature of 35°...thus it would have been prima facie obvious to combine the teachings of Esposito et al. with the teachings of Giuliani et al."

Applicants respectfully submit that Giuliani discloses LTK63, which is a genetically attenuated toxin that has not been treated with formalin. As discussed above, Applicants submit that it would be unpredictable whether the process of formalin treatment of LTR72 would result in an attenuated toxin that is equivalent to LTK63, which is completely nontoxic and has adjuvanticity inferior to that of wild-type LT and LTR72. Thus, this reference teaches that genetic attenuation of toxins achieves greatly reduced toxicity and does not suggest the necessity of chemical attenuation. Therefore, one skilled in the art would not have been motivated by Esposito to chemically attenuate the recombinant toxins of Giuliani.

For the reasons set forth above, Applicants respectfully submit that one of skill in the art would not have contemplated further detoxification of LTR72 and would therefore have had no motivation to choose the formalin treatment taught by Esposito to combine with the disclosure of Giuliani. Thus, since Applicants identified the unexpected result of producing a highly attenuated toxins while retaining adjuvant activity, Applicants submit that a *prima facie* case of obviousness has not been established. Withdrawal of the rejection is respectfully requested.

Conclusion

In summary, for the reasons set forth herein, Applicants maintain that the claims clearly and patentably define the invention and respectfully request that the Examiner withdraw all rejections and pass the application to allowance. If the Examiner would like to discuss any of the issues raised in the Office Action, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

No fee is deemed necessary in connection with the filing of this paper. However, the Commissioner is hereby authorized to charge any fees that are required, or credit any overpayments to Deposit Account No. 07-1896 referencing the above-identified attorney docket number.

Respectfully submitted,

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Antony M. Novom, J.D.
Registration No. 45,517
Telephone: (858) 638-6641
Facsimile: (858) 677-1465

DLA PIPER LLP (US)
4365 Executive Drive, Suite 1100
San Diego, California 92121-2133
USPTO CUSTOMER NO. 28213

Attachments: Exhibit A Ward et al., PubMed Abstract of Infect Immun. 1999, 67(10): 5124-32.
Exhibit B Tierney et al., PubMed Abstract of J Infect Dis. 2003, 188(5): 753-8.
Exhibit C Kende et al., PubMed Abstract of Vaccine. 2007, 25(16): 3219-27.
Exhibit D Chen et al., PubMed Abstract of Vaccine. 2002, 20(21-22): 2671-9.
Exhibit E Tamura et al., PubMed Abstract of Jpn J Infect Dis. 2000, 53(3): 98-106.